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α -ADDUCIN POLYMORPHISM ASSOCIATED WITH INCREASED RISK OF ADVERSE CARDIOVASCULAR OUTCOMES: RESULTS FROM INVEST-GENES

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Abstract

Background—The α -adducin (*ADD1*) Gly460Trp polymorphism has been associated with hypertension and response to diuretic therapy, but controversy exists.

Methods—The present study was conducted to prospectively investigate the relationship between the *ADD1* Gly460Trp polymorphism, diuretic use, and adverse cardiovascular outcomes among 5,979 hypertensive coronary artery disease patients, who participated in the International Verapamil SR-trandolapril Study (INVEST) and provided genomic DNA. The primary outcome was defined as first occurrence of nonfatal stroke, nonfatal myocardial infarction, or all-cause death. Secondary outcomes were the components of the primary outcome. Ancestry informative markers were used to control for population stratification.

Results—In Blacks, *ADD1* variant carriers were at higher risk for a primary outcome event than wild-type homozygotes (adjusted hazard ratio (HR) 2.62; 95% confidence interval (CI) 1.23–5.58; $p = .012$), with a similar trend in Whites and Hispanics, albeit a smaller magnitude of effect (adjusted HR 1.43, 0.86–2.39 in Hispanics; 1.24, 0.90–1.71 in Whites). Secondary outcome analysis showed that the all-cause death was driving the differences in primary outcomes by genotype. There was no interaction between the *ADD1* polymorphism and diuretic use for either primary outcome or secondary outcomes.

Conclusions—In hypertensive patients with coronary artery disease, black *ADD1* variant carriers showed a 2.6-fold excess risk for a primary outcome event and an 8-fold increase risk of death. White and Hispanic *ADD1* variant carriers showed an increased but nonsignificant excess risk. However, the effect of diuretic use on risk of cardiovascular outcomes did not vary by *ADD1* carrier status.

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Keywords

pharmacogenetics; *ADD1*; diuretics; cardiovascular outcomes

INTRODUCTION

Hypertension affects over a quarter of the world's population and increases risk for both cardiovascular and kidney disease.¹ Identification of patients at risk as well as treatment of patients remain inadequate.² Genetic variation is likely an important factor influencing both susceptibility to hypertension, the adverse outcomes association with hypertension and response to antihypertensive drugs.³ A pharmacogenetics-based treatment approach, i.e. consideration of genetic variation in the individual selection of antihypertensive agents, may allow improved blood pressure control and reduction in adverse sequelae by maximizing the likelihood of benefit while minimizing the likelihood of harmful effects of a given treatment.⁴

The α -adducin (*ADD1*) gene has emerged as both a potential risk factor for hypertension, as well as candidate for effect modification of antihypertensive therapy.⁵ Adducin is a ubiquitously expressed heterodimeric cytoskeleton protein that promotes the binding of spectrin with actin and may modulate a variety of other cell functions such as ion transport.⁶ Animal models established that single nucleotide polymorphisms (SNPs) in the *ADD1* gene lead to increased tubular sodium reabsorption and hypertension.⁷ In humans, a guanine to thymine SNP at nucleotide 614 in exon 10 of the *ADD1* gene (rs4961) leads to a glycine (*Gly*) to tryptophan (*Trp*) change at amino acid position 460. This polymorphism has been associated with elevated untreated blood pressure (BP),⁸ hypertension,⁹ salt sensitivity¹⁰ and response to diuretic therapy.¹¹ However, as with other candidate genes investigated in multifactorial polygenic diseases, the evidence remains inconsistent between studies and across populations.

To date, few studies have investigated the association between the *ADD1 Gly460Trp* polymorphism and adverse cardiovascular outcomes, and results are conflicting.^{11, 12} A population based case control study of pharmacologically treated hypertensives suggested no significant association of the *460Trp* allele with cardiovascular outcomes (first nonfatal stroke or first nonfatal myocardial infarction, MI).¹¹ However, diuretic use was associated with a significant reduction in CV outcomes only among *ADD1* variant carriers with no such benefit seen in wild-type homozygotes, which if true could have important implications regarding genotype-guided therapy with diuretics. In a recently published outcome trial, the Genetics of Hypertension-Associated Treatment Study (GenHAT), the *ADD1 Gly460Trp* polymorphism was not an important modifier of antihypertensive treatment on cardiovascular risk (coronary heart disease, CHD), nor was an effect on adverse outcomes noted in this study.¹² However, the authors reported a significant sex**ADD1**treatment interaction whereby female variant carriers treated with diuretics had an increased risk of CHD not evident in men. Given the disparity in findings of these two studies, additional data are needed to help define the potential role of the *ADD1* polymorphism relative to outcomes associated with diuretic therapy.

To prospectively investigate the association of the *ADD1 460Trp* allele with cardiovascular outcomes and to examine its interaction with diuretic therapy, the present study was conducted within a subset of patients from a large clinical trial where antihypertensive therapy was randomized and blood pressure was well controlled.

METHODS

Study population and interventions

The present study was pre-specified as part of INVEST-GENES, the GENetic Substudy of the International VErapamil SR-trandolapril Study (INVEST). After an extensive cardiovascular history and physical exam, the INVEST randomly assigned 22,576 CAD patients ≥ 50 years old to either a verapamil SR or an atenolol-based multidrug antihypertensive strategy. Trandolapril and/or hydrochlorothiazide (HCTZ) were added if needed for BP control. Between 1997 and 2003, 61,835 patient-years follow-up were accumulated. The primary outcome was defined as all-cause death, nonfatal MI or nonfatal stroke. All components of the primary outcome were fully adjudicated by an independent adjudication committee.¹³ Secondary outcomes were the individual components of the primary outcome. Further details have been published.^{13, 14} Genetic samples were collected on 5,979 subjects from 184 sites in mainland US and Puerto Rico. The INVEST-GENES was approved by the institutional review board (IRB) of the University of Florida, which acted as the central IRB for all participating sites, and written informed consent for participation in the genetic substudy was provided by each patient.

Hypotheses

We hypothesized that compared to *ADD1* wild-type homozygotes, carriers of one or two copies of the *ADD1* 460*Trp* allele would (1) be at higher risk of experiencing a primary outcome event and (2) have a greater reduction in risk of a primary outcome event when treated with diuretics.

Drug exposure for add-on drugs

Drug exposure to primary strategy drugs (atenolol and verapamil SR) was defined by INVEST randomized treatment strategy. Patients were only exposed to added agents after failure to meet BP goals or for compelling indications for trandolapril. Therefore, exposure to HCTZ or trandolapril as part of the INVEST treatment protocol was operationalized as a time-varying covariate and coded 0 before exposure and 1 from the date of the first prescription to the end of the study follow up or event.

Genotyping

Genomic DNA was isolated from buccal genetic samples and normalized to 20ng/ μ l. Genotyping was performed by polymerase chain reaction (PCR) followed by Pyrosequencing® (Pyrosequencing, Uppsala, Sweden).¹⁵ The genotype and primary event data have been deposited in the Pharmacogenomics Knowledge Base (www.pharmgkb.org; accession Nos. PS205467 and PS205547).

Ancestry informative markers

To control for potential population stratification in our racially and ethnically diverse population, we used a panel of 87 autosomal ancestry informative markers (AIMs) that show large allele frequency differences across three parental populations (West Africans, Indigenous Americans, and Europeans).¹⁶ These AIMs were selected to be distributed across the genome and to be distantly interspaced to give independent association with the disease and genetic background. These 87 AIMs were genotyped using either allele-specific PCR with universal energy transfer labeled primers¹⁷ or competitive allele specific PCR at Prevention Genetics (Marshfield, Wisconsin).

Statistical Analysis

Due to the rarity of the *Trp460Trp* genotype (2.7%, 152 of 5,661), study subjects were classified as either *ADD1* wild-type homozygotes (*Gly460Gly*) or as *ADD1* variant carriers

(*Gly460Trp* or *Trp460Trp*). Baseline characteristics between *ADD1* variant carriers and wild-type homozygotes were compared using t-test for continuous and Chi-squared test for categorical variables. Hardy-Weinberg equilibrium (HWE) within each race/ethnic group was tested with Chi-squared test with one degree of freedom.

Kaplan Meier curves were estimated to compare time to first primary outcome and secondary outcome by *ADD1* variant carrier status as well as *ADD1* variant carrier status and diuretic use. Maximum likelihood was used to estimate each patient's individual genomic ancestry proportions¹⁶ on these three parental ancestries and two of these three terms were included in statistical models to control for ancestry.

For hypothesis 1, the effect of *ADD1* variant carrier status on primary outcome events was investigated within each ethnicity group using a Cox proportional hazards (PH) model adjusting for ancestry information. AIMS were included as percentage of Indigenous American ancestry and African ancestry. In addition to INVEST treatment strategy, the use of HCTZ and/or trandolapril and AIMS, the adjusted model controlled for five prespecified INVEST covariates³¹ (age, sex, ethnicity, previous MI, and prior heart failure), and other potential confounders selected by a backwards stepwise selection procedure ($p = 0.05$): history of smoking, diabetes, renal insufficiency, body mass index (BMI), baseline SBP. Time-varying exposure was used for HCTZ and trandolapril. Additionally, the Cox PH model was used to calculate adjusted hazard ratios (HR) and confidence intervals (CI) for primary outcome for *ADD1* variant carrier status by sex, age ($>70y$ vs. $\leq 70y$), SBP ($>160\text{mmHg}$ vs. $\leq 160\text{mmHg}$), treatment strategy, smoking status, and for secondary outcomes.

The gene-diuretic interaction (hypothesis 2) was tested as the interaction between *ADD1* variant carrier statuses with time-varying exposure to diuretics in the Cox PH model. The interactions of the *ADD1* variant with sex, ethnicity, age, SBP, and smoking status were tested using an analogous approach. In addition, differences in the *ADD1**treatment interaction by sex were tested as the *ADD1**treatment*sex interaction term.

Analysis of covariance was used to test for differences in the number of strategy antihypertensive drugs, total antihypertensive drugs, as well as BP at the end of follow-up between carriers of the *ADD1* variant and wild-type homozygotes controlling for significant covariates. SAS version 9.1.3 (SAS institute, Cary, NC) was used for data management and statistical analysis.

RESULTS

Study population and baseline characteristics

Baseline demographic and study data for the INVEST-GENES cohort by *ADD1 Gly460Trp* polymorphism are summarized in Table 1 and Table 2. Patients in the INVEST-GENES included a large proportion of elderly, diabetic and female patients that were ethnically diverse with 47% Hispanics, 41% whites and 11% blacks. The overall primary outcome event rate in the INVEST-GENES was 4.6%, and 68.0% of patients used diuretics at some time during the study. For patients exposed to diuretics, average time of exposure was 25.0 (SD 11.1) months, with no difference between *ADD1* variant carriers and wild-type homozygotes.

Genotypes for the *ADD1* polymorphism were determined for 5,661 (94.7%) of the 5,979 INVEST-GENES patients and these included all patients who experienced a primary outcome event. Importantly, patients with missing genotype data show no statistically or clinically significant differences from the 5,979 patients for whom genetic samples were collected (P -values for all patient characteristics listed in Table 1 $>.5$).

Baseline characteristics and study drugs of *ADD1* variant carriers were generally comparable to those of wild-type homozygotes as detailed in Table 1 and Table 2. There are small statistically significant differences in age, angina prevalence, and history of CABG between *ADD1* variant carriers and wild-type homozygotes. All of these variables were included in our multivariable modeling in order to adjust for any contributions to differences in outcomes these differences may have led to.

Among Hispanics and Blacks, the *ADD1* variant was in HWE, but not among Whites ($p = .019$). When only Whites with greater than 85% of European ancestry were included, the genotype frequencies did not deviate from HWE, suggesting that the observed deviation from HWE was due to a small portion of the sample with a high degree of non-European admixture. Of the 5,661 patients with genotype data, 1,773 (31.3%) were carriers of the *ADD1* variant (Table 3).

INVEST Treatment Effects

Consistent with the full INVEST study population,¹³ there was no significant difference in primary outcome events between treatment strategies for patients included in the INVEST-GENES (HR 1.14; 95% CI 0.89–1.46, $p = 0.30$).

Association of *ADD1* carrier status with risk of clinical outcomes

The incidences of primary and secondary outcome events by *ADD1* variant carrier status within each race/ethnicity group, as well as HRs for variant carrier status are shown in Table 4. Black *ADD1* variant carriers had a 2.4-fold increased risk for a primary outcome event and significantly shorter time to primary outcome event than non-carriers ($p = 0.013$). A similar trend was seen in Hispanics and Whites, although was not significant (Hispanics, $p = 0.12$; Whites: $p = 0.28$) (Table 4).

After adjusting for AIMs and covariates, *ADD1* variant carrier status remained associated with significant excess risk for a primary outcome event in Blacks (2.62 [1.23–5.58], $p = 0.012$). Adjusted HRs and CIs in Whites and Hispanics are shown in Table 4 (1.43 [0.86–2.39] in Hispanics; 1.24 [0.90–1.71] in Whites).

Given that the findings in the composite primary outcome event may represent a signal for an effect in one or two but not all of the components of the primary outcome, we next investigated the influence of variant carrier status on all-cause death, nonfatal myocardial infarction and nonfatal stroke (Table 4). These data suggest that the effect of *ADD1* genotype on the primary outcome is primarily driven by differences in all-cause death by genotype. Kaplan Meier curves for all-cause death in each race/ethnicity group are shown in Figure 1. The adjusted model for *ADD1* variant carrier status showed an almost 8-fold excess risk of all-cause death in Blacks ($p = 0.016$), 2-fold higher risk in Hispanics ($p = 0.05$) and a trend toward higher risk in Whites (46%; $p = .16$). No significant difference in the risk of nonfatal MI or nonfatal stroke in any of the three race/ethnicity groups was observed.

Interactions

Time to primary outcome event was not significantly different between users and non-users of diuretics regardless of *ADD1* variant carrier status in any of the three race/ethnicity groups. Given the consistency of this finding in the race/ethnicity groups, we combined them, and the combined Kaplan Meier curve by diuretic therapy and *ADD1* variant carrier status is shown in Figure 2 ($p = 0.86$ and 0.99 for variant carriers and non-carriers, respectively). Accordingly, the interaction between *ADD1* carrier status and diuretics was not significant in the adjusted Cox PH model ($p = 0.47$).

When calculated individually for all-cause death, nonfatal stroke or nonfatal MI (the outcome used in a previous study showing an effect modification of diuretics by *ADD1*), the interaction term remained not significant ($p > 0.44$).

Additionally, the interactions of *ADD1* carrier status with sex, age, SBP, history of smoking, and INVEST treatment strategy (i.e. verapamil SR- versus atenolol-based strategies) were tested individually in the Cox PH model. None of these interaction terms reached statistical significance (all p -values > 0.25). There was no evidence for *ADD1**diuretic*sex interaction (1.165 [0.71–1.91], $p = 0.547$).

Secondary analyses

Adjusted HRs for *ADD1* variant carrier status stratified by sex, age, SBP and smoking status were also calculated. In all subgroups, the point estimates for variant carriers were greater than 1, although due to power limitations in the subgroups, not all subgroups were significant. These data suggest consistency in the risk for *Typ460* carrier status across subgroups.

Adjusted for significant covariates, there was no difference between the number of strategy drugs, overall drugs, or DBP at the end of follow-up between carriers and non-carriers of the *ADD1* variant (1.88 vs. 1.92, $p = 0.19$ for strategy antihypertensive drugs; 2.61 vs. 2.71, $p = 0.49$ for total antihypertensive drugs; 76.9 vs. 76.9, $p = 0.93$ for DBP).

DISCUSSION

This study prospectively investigated the association of the *ADD1 Gly460Trp* polymorphism with cardiovascular outcomes in CAD patients with hypertension. In black hypertensive CAD patients we found that *ADD1* variant carriers showed 2.62 times higher risk for a primary outcome event and an 8-fold increased risk of death. Among Hispanics, there was a non-significant 43% increased risk of a primary outcome event and 2-fold increased risk of death ($p = 0.05$). Among Whites, no significant effect was observed, although the point estimates for the primary outcome and death exceeded 1.

Over the past decade, the *ADD1 Gly460Trp* polymorphism has emerged as a potential determinant of blood pressure and cardiovascular outcomes. However, results from different studies are conflicting. Our data did not confirm previously reported interactions between *ADD1* genotype and SBP^{18, 19}, age¹⁹, and smoking¹⁹. These studies did not show a main effect and differences in findings may be explained by differences in study end points, ethnicity, age range, and BP range of participants.

The INVEST-GENES also did not show evidence for a pharmacogenetic association between the *ADD1* variant and diuretic use. While a retrospective case-control study suggested that only *ADD1* variant carriers would benefit from diuretic therapy,¹¹ our findings show no difference in the effect of diuretics on primary outcome events between *ADD1* variant carriers non-carriers. Our results were unchanged when we evaluated the effect of the diuretic**ADD1* interaction on the combined incidence of non-fatal stroke or non-fatal MI, the outcome used in the former study. A potential explanation for differing findings could relate to differences in study design and diuretic treatment patterns. While the INVEST protocol directed HCTZ as 2nd and 3rd steps for patients not adequately controlled with atenolol or verapamil-SR plus trandolapril alone, diuretic therapy in the other study was not utilized in any specified fashion, and the report provides minimal information on the percent of patients on diuretic monotherapy, when diuretics were added to the drug regimen, or adherence to such therapy. In contrast, use of diuretics in the INVEST was protocol-driven, the precise timing for addition of diuretics is known (and included in the modeling), and the high level of BP control achieved in the INVEST (e.g. $> 70\%$ of patients achieving $< 140 / < 90$ mm Hg¹³) suggests good adherence to therapy.

Given the high level of BP control achieved in the INVEST, differences in BP control might also explain differences in findings between the two studies. However, the other study reported no change in the size of the interaction when controlling for SBP and did not find evidence for interaction between diuretic use and the *ADD1* variant on SBP.

We recognize that analyses using time-varying covariates may be biased when (1) there exists a time-dependent covariate that is a risk factor for, or predictor of, the event of interest and also predicts subsequent treatment; and (2) past treatment history predicts subsequent risk factor level.²⁰ In our study, these conditions may be met because SBP levels over follow-up is a risk factor for primary outcome, could plausibly be associated with diuretic use, and is likely affected by previous treatment with diuretics.

Our results confirmed the race-adjusted results from the Genetics of Hypertension-Associated Treatment Study (GenHAT) that the *ADD1* variant does not modify the effect of the diuretic therapy on cardiovascular risk. However, no effect of *ADD1* on outcomes was observed in GenHAT, in this study we observed a strong *ADD1* main effect on primary outcome among Blacks.¹² One possible explanation is that the patients in INVEST-GENES were higher risk population than those in GenHAT, i.e. patients with documented CAD and heart failure were included in INVEST but excluded in GenHAT.^{13, 14, 21} Differences in race/ethnicity in the two studies may have also contributed, although this seems less likely since GenHAT also included a highly diverse population.

When AIMs were used to control for the population stratification, the results for both *ADD1 Gly460Trp* main effect and interaction with the diuretic therapy did not change compared to the analysis using ethnicity term alone, indicating that population stratification was not a confounding factor for our study.

In summary, the INVEST-GENES results add to the current knowledge base about the *ADD1 Gly460Trp* polymorphism in cardiovascular disease and suggest that it may represent an important risk factor for all-cause death among Blacks with hypertension and coronary artery disease. No differences in the response to diuretic therapy were observed between *ADD1* variant carriers and non-carriers. This suggests that *ADD1* genotype guided therapy with diuretics, as proposed by a recent pharmacoeconomic analysis²² may not be a clinically or economically useful method to improve antihypertensive therapy. Additional studies are warranted to further clarify the role of variation of the *ADD1* gene in cardiovascular disease and to evaluate its potential role as a component of future risk stratification scores.

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Figure 1. Kaplan Meier curves for all-cause death by *ADD1* variant carrier status within each race/ethnicity group

A. Blacks; B. Hispanics; C. Whites. Color: black: *ADD1* variant carrier; gray: *ADD1* wild-type homozygotes.

No. at risk

<i>Trp460-Diur.</i>	1195	1179	1173	1167	1144	821	479	174	126
<i>Gly460Gly-Diur.</i>	2654	2621	2612	2598	2556	1804	1047	407	293
<i>Trp460-No Diur.</i>	578	573	568	562	552	391	189	64	44
<i>Gly460Gly-No Diur.</i>	1234	1219	1212	1207	1193	847	383	105	57
Overall	5661	5592	5565	5534	5445	3863	2098	750	520

Figure 2. Kaplan Meier Curves for primary outcome event by *ADD1* variant carrier status and diuretic use

Abbreviations: *Trp460* indicates *ADD1* variant carrier

Table 1Baseline Characteristics by *ADD1* carrier status*

	INVEST-GENES with complete <i>ADD1</i> genotypes (n=5661)	<i>ADD1</i> variant carrier (n=1773)	Wild-type homozygotes (n=3888)	<i>P</i> [†]
Demographics and social history				
Age, mean (SD), y	66.2 (9.7)	66.6 (9.8)	66.0 (9.7)	0.04
>70y	1931 (34.1)	644 (36.3)	1287 (33.1)	0.02
Women	3167 (55.9)	984 (55.5)	2183 (56.15)	0.65
Ethnicity				
White	2302 (40.7)	859 (48.4)	1443 (37.1)	<0.0001
Black	654 (11.5)	109 (6.1)	545 (14.0)	
Hispanic	2671 (47.2)	792 (44.7)	1879 (48.3)	
Other	34 (0.6)	13 (0.7)	21 (0.5)	
BMI, mean (SD), kg/m ²	29.4 (5.6)	29.4 (5.9)	29.3 (5.4)	0.61
Smoking				
Past	2339 (41.3)	758 (42.8)	1581 (40.7)	0.14
within last 30d	578 (10.2)	180 (10.2)	398 (10.2)	0.92
Never	3322 (58.7)	1015 (57.2)	2307 (59.3)	
Past medical history at study entry				
Myocardial Infarction	1319 (23.3)	436 (24.6)	883 (22.7)	0.12
Angina pectoris	4207 (74.3)	1263 (71.2)	2944 (75.7)	<0.0001
CABG ≥1 month ago	808 (14.3)	282 (15.9)	526 (13.5)	0.02
Stroke/TIA	388 (6.9)	121 (6.8)	267 (6.9)	0.95
Left ventricular hypertrophy	849 (15.0)	267 (15.1)	582 (15.0)	0.93
Unstable angina ≥1 mo ago	548 (9.7)	184 (10.4)	364 (9.4)	0.23
Arrhythmia	394 (7.0)	136 (7.7)	258 (6.6)	0.15
Heart failure (class I–III)	192 (3.4)	69 (3.9)	123 (3.2)	0.16
Peripheral vascular disease	627 (11.1)	203 (11.5)	424 (10.9)	0.54
Diabetes	1344 (23.7)	405 (22.8)	939 (24.2)	0.28
Hypercholesterolemia	3087 (54.5)	954 (53.8)	2133 (54.9)	0.46
Renal impairment	88 (1.6)	26 (1.5)	62 (1.6)	0.71
Cancer	229 (4.1)	73 (4.1)	156 (4.0)	0.85
Medication				
Aspirin/other antiplatelet therapy	2592 (45.8)	857 (48.3)	1735 (44.6)	<.01
Other NSAIDs	1341 (23.7)	406 (22.9)	935 (24.1)	0.34
Antidiabetic medication	1344 (23.7)	405 (22.8)	939 (24.2)	0.28
Any lipid-lowering agent	2047 (36.2)	653 (36.8)	1394 (35.9)	0.48

Abbreviations: CABG, coronary artery bypass graft; TIA, transient ischemic attack; NSAID, non-steroidal anti-inflammatory drug.

* Data are presented as number and percentage unless otherwise indicated

[†] T-test for continuous and Chi-squared test for categorical variables.

Table 2

Baseline Blood Pressure and Antihypertensive Medications, study drugs and intermediate outcomes.

	<i>ADD1</i> variant carrier (n=1773)	Wild-type homozygotes (n=3888)	<i>P</i>
Heart rate, mean (SD), beats/min	74.9 (9.6)	74.8 (9.6)	.96
Blood pressure at study entry, mean (SD), mm Hg			
Systolic	148.5 (18.0)	147.8 (18.3)	.18
Diastolic	85.1 (10.7)	85.6 (10.6)	.12
No. (%) with BP in control at study entry			
Systolic	414 (26.4)	949 (27.4)	.49
Diastolic	959 (61.2)	2008 (57.9)	.03
Both	374 (23.9)	827 (23.9)	.98
Study arm and added drugs			
Verapamil SR-based strategy	865 (48.8)	1901 (48.9)	.94
HCTZ	1195 (67.4)	2654 (68.3)	.52
Person-time exposed to HCTZ, mean (SD), months	24.5 (10.9)	25.2 (11.1)	.06
Trandolapril	1314 (74.1)	2885 (74.2)	.94
No. of strategy drugs at end of follow-up, mean (SD)	1.87 (1.06)	1.92 (1.06)	.11
Total No. of antihypertensive drugs at end of follow-up, mean (SD)	2.66 (1.31)	2.68 (1.36)	.64
Intermediate outcomes			
Percent of visits with BP in control, mean (SD)	61.0 (26.5)	60.5 (27.2)	.53
SBP at 12 months, mean (SD), mm Hg [§]	133.2 (16.1)	133.4 (16.5)	.70
DBP at 12 months, mean (SD), mm Hg [§]	77.7 (9.0)	78.1 (9.6)	.09
BP in control at 12 months [§] , No. (%)	1023 (62.5)	2175 (59.8)	.22
SBP at 24 months, mean (SD), mm Hg [§]	131.9 (15.2)	131.7 (15.5)	.68
DBP at 24 months, mean (SD), mm Hg [§]	77.3 (8.8)	77.3 (9.1)	.92
BP in control at 24 months [§] , No. (%)	984 (65.3)	2141 (64.1)	.76

Abbreviations: HCTZ, hydrochlorothiazide; SBP, systolic BP; DBP, diastolic BP.

* Data presented as number and percentage unless otherwise indicated.

[‡]BP in control as SBP<140 mm Hg and DBP<90 mm Hg[§]N=patients at risk at 12 and 24 months, respectively.

Table 3*ADD1* Gly460Trp Genotype Frequency for INVEST-GENES and by Ethnicity

	<i>Trp/Trp</i>	<i>Gly/Trp</i>	<i>Gly/Gly</i>	Trp allele frequency
INVEST-GENES	152 (3%)	1621 (29%)	3888 (69%)	17%
White	77 (3%)	782 (34%)	1443(63%)	20%
Black	8 (1%)	101 (15%)	545 (83%)	9%
Hispanic	64 (2%)	728 (27%)	1879 (70%)	16%
Other	3 (9%)	10 (29%)	21 (62%)	23%

Table 4

Adjusted and unadjusted hazard ratios for *ADD1* carrier status

	Variant carrier [‡] (n=1773)	Wild-type homozygotes [‡] (n=3888)	Unadjusted HR (95% CI) for variant carrier status	Adjusted HR [†] (95% CI) for variant carrier status
Primary outcome event				
	Whites (n=2,302)	92 (6.4)	1.19 (0.87–1.63)	1.24 (0.90–1.71)
	Hispanics (n=2,608)	24 (3.0)	1.50 (0.90–2.49)	1.43 (0.86–2.39)
	Blacks (n=654)	11 (10.1)	2.39 (1.17–4.85)*	2.62 (1.23–5.58)*
	Other (n = 34)	0	NA	NA
Secondary outcomes				
	Whites	25 (2.9)	1.31 (0.78–2.23)	1.46 (0.86–2.49)
	Hispanics	15 (1.89)	2.14 (1.07–4.30)*	2.01 (1.00–4.03)*
	Blacks	5 (4.6)	4.73 (1.44–15.54)*	7.95 (1.46–43.19)*
	Whites	19 (2.2)	0.85 (0.49–1.48)	0.78 (0.44–1.39)
Nonfatal MI	Hispanics	4 (0.5)	1.08 (0.33–3.51)	1.02 (0.31–3.36)
	Blacks	3 (2.7)	1.77 (0.48–6.54)	1.82 (0.47–7.06)
	Whites	26 (3.0)	1.67 (0.97–2.88)	1.71 (0.99–2.95)
Nonfatal stroke	Hispanics	5 (0.6)	0.92 (0.33–2.59)	0.90 (0.32–2.54)
	Blacks	4 (3.7)	1.85 (0.59–5.81)	1.91 (0.58–6.36)

Abbreviations: HR, hazard ratio; CI, confidence interval; CV, cardiovascular; MI, myocardial infarction.

[‡] number (percentage)

* p < 0.05

[†] adjusted for age, gender, race/ethnicity, AIMS, history of MI and heart failure, treatment strategy, diabetes, BMI, smoking, renal insufficiency.